Clarifications to Bidder’s Questions:

Microbial Communities as Indicators of Non-persistent Pesticides

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| 1 | Is it possible to outsource the sequencing element to do the 16S and ITS sequencing and provide us with the raw data? | Yes, there is no problem to outsource the sequencing, provided that all costs are agreed in advance and budgeted for in your tender |
| 2 | Can you please clarify if the 5 treatments are 4 pesticides + control, or 5 pesticides + control? | Our original specification asks for 5 pesticide treatments plus control. However, if this is not possible within reasonable costing and timescales, we will consider bids that deliver tests with only 4 pesticides. |
| 3 | Is there a particular format or a specific template that you would like the responses submitting in? The RFQ document doesn’t appear to have a proforma for completion. | There is no standard form, but we would advise you to look at the following sections of the Request for Quotation document: Evaluation methodology (page 17): this sets out the way in which we will evaluate your tender, and we’d recommend using the “Evaluation Criteria” as headings for your submission and make sure you address the requirements in the “what we’re looking for” column, to match our evaluation criteria. Please also supply the information in the sections headed “information to be returned” (page 21), and in Annex 1 on page 23. |
| 4 | To confirm 3 soil types, 5 treatments, 5 replicates so there will be 75 pots? | As stated above, the original specification asks for 6 treatments (5 pesticides plus a control). However, the proposal also asks that these are sampled twice. If sampling is destructive, you will need to double the replicates. Sampling mesocosms without disturbing them, and thereby affecting the results of a later sampling, may be difficult, but if you think you can achieve this, it may be a way to reduce the number of replicates. |
| 5 | Is there any guidance to the pot size, crop type or number grown in the plant, environmental conditions? | It’s up to you to decide this, but in past experiments we have grown a single winter wheat plant in a smallish plant pot. You would need to manage smaller mesocosms carefully to avoid drying/edge effects. However, larger mesocosms will require the collection of more soil material, which may be harder to obtain, collect, process or transport. |
| 6 | Is winter wheat the only candidate for the crop or any (arable?) crop? We have a grow tent, depends on the size of the trials we might be able to do the pot trials indoor. | The reason for planting a plan in the mesocosms is to supply the soil with some ongoing C input through root exudates, to simulate normal soil management and maintain microbial activity. Wheat was suggested because it both exudes exudates and is potentially mycorrhizal. We would advise against using a non-mycorrhizal plant (brassicas, beets), because this might limit the understanding we can gain from the experiment on pesticide impacts on mycorrhizal fungal communities. |
| 7 | Is any nutrient or physical sampling required on the treatments? | We don’t require this, but if you can at least characterise the texture by hand that would be great. Please also record the locations of the collection sites. We would recommend retaining samples of the test soils, and if your budget can stretch, including nutrient / SOM /pH /texture analysis. However, if budgets and time don’t allow for this, retaining the soils so that NE can pay for their analysis separately would also be an acceptable option. |
| 8 | Are there any other parameters in addition to 16S/ITS that are going to be looked at? Quantification of the pesticide remains would be useful. And since metabarcoding is not quantitative it would be good to combine it with quantification using microscopy. | I’m not sure the scale of the funding available would cover this additional assessment. The aim of the project is to generate data comparable to that collected from field sites by the EES, which is carrying out metabarcoding only, and is not recording pesticide residues. However, if you able to commit to retain samples in suitable storage (frozen?), for a year or so, we may be able to analyse the samples for pesticide residues/degradation products, in the future. |
| 9 | Would it be possible to have an online meeting to confirm these points? | Sorry – all information about the contract has to be shared with all potential contractors, so we can’t really hold individual meetings. |
| 10 | Would Natural England be open to receiving proposals to include a literature review alongside a smaller microcosm study (fewer treatments and/or reduced replication) and for the project to be delivered over a longer time period? | We would consider proposals with fewer replicates or treatments, but the project must fit within the timescales available, as this is when the budget is available. We would expect, however, for “final tweaks” to be made to some project outputs following delivery at the end of march, provided the project has been substantially delivered. |
| 11 | Regarding the rapid literature review: Could you clarify the expected depth of this review, including word length and the approximate number of papers to cover? Would a table summarising each compound and its known effects be acceptable? Given the timescale and contract value, this component could require substantial resources. | The literature review would be limited to those pesticides chosen for the experiment, and would be a rapid review of maybe 4-5 papers maximum, which would indicate the possible likely microbial responses. If existing reviews have covered this topic already, then a quick summary of the findings, and a reference to that review, should be sufficient. The aim of the review is to seek “proof of concept” that the pesticides applied have been observed to have impacts on microbial communities. If they are clearly demonstrated to not have impacts, this might rule them out from the study. |
| 12 | Regarding the selection of soil types from established organic farms: Have these been pre-selected by NE? Are the locations known (for travel costing purposes), and is prior permission from the landowners to collect samples already obtained? Gaining these agreements could be time-sensitive and potentially limit project feasibility. | NE are happy to suggest and contact farmers to request sampling sites, using our existing network of contacts. However, contractors may wish to explore possible sites among their own contacts or sites. I would assume that the sites are widely distributed across England, for costing T&S (e.g. one in SW, one in the E of England, one in the N of England), and base travel costs on this. |
| 13 | Regarding the chemical treatments: Is the intention to conduct a single experiment with a mixture of all five pesticide groups (i.e., two treatments—mixture/control, with five replicates, sampled at three time points, across three soil types, resulting in 90 samples total for sequencing)? Or is it five separate treatments, one for each pesticide group plus a control (six treatments in total, five replicates, sampled at three time points, across three soil types, resulting in 270 samples total for sequencing)? | This would be very interesting, but perhaps would work well as a follow on, larger experiment, once we’ve demonstrated impacts (if any) of applications of single pesticides. The reality in the field will be that microbes may be exposed to repeated applications of a range of different pesticides, which may change the responses observed |
| 14 | Regarding pot management: As mentioned, is it a requirement to grow a winter wheat plant in each pot? This could increase variability and complicate analysis, as it may be more challenging to distinguish responses from free-living versus rhizosphere-associated microbial communities. Additionally, there is a risk of uneven germination across replicates, which could complicate comparisons. Variability in plant growth and root surface area may also increase heterogeneity between replicates. | The aim of the project is to help us, in the future, interpret EES field soil sampling data, and field samples will be likely to have growing plants. We would be interested therefore in both rhizosphere and bulk soil microbes, and the combined impact of pesticides on both groups, simultaneously. It would be possible to germinal the plants before application of the pesticides, so that you can be confident that the replicate pots all have a successfully germinated plant in them. |
| 15 | Regarding the project timeline: From our perspective, conducting three separate batches of sequencing within the project’s short timescale would be neither cost-effective nor feasible. Would it be acceptable to carry out DNA extraction, PCR, and sequencing for all samples at the end of the experiment? | Yes, provided that you are confident that the DNA sequencing will be completed by the end of the experiment and that there will be enough time to collate and present the results. |
| 16 | Regarding the final reporting timeline: While it may be feasible to complete the experimental setup and sampling by March, the detailed analysis of microbial communities via metabarcoding and the final report would likely require more time. Would an extended deadline of June 2025 for final reporting be acceptable? | We appreciate that this project will aim mainly to deliver the basic results, and we hope to analyse these in the future either in-house or through a future contract. We can’t, however, set up a contract extension, so this project aims just at delivering results within the timescale available. |
| 17 | Regarding EES 16S & ITS data methodologies: Will detailed methods be provided to ensure consistency across datasets? | We will ask for advice from colleagues more expert on genetic data processing to advise on this once the contract is available, but we would want the data management protocol to be as compatible as possible with that being used by the England Ecosystems Survey, where the genetic work is being carried out by FERA. |
| 18 | Regarding community variables for analysis: Do you have a defined list of microbial community variables that you would like to test for differences? Potential reporting metrics include taxonomic composition (relative abundance at a predefined taxonomic cutoff—e.g., phyla, order, class—and significant differences between controls and treatments tested via Kruskal-Wallis), alpha diversity (taxa richness and Shannon diversity at OTU/ASV level, also tested for significance via Kruskal-Wallis), and beta diversity (Bray-Curtis distance visualized by NMDS, with community-level significance assessed via PERMANOVA). Would these metrics and methods be acceptable as deliverables? | I would suspect that taxonomic composition (visualised by NMDS) would be the most useful characteristic to report on the impacts of pesticides, since we’re looking for community “signatures” that indicate exposure to the pesticides, and if there are changes in groups of taxa with know ecological functions. More general descriptors of communities (alpha diversity, richness etc.) might also be useful in understanding impacts, but are a lower priority, since the same diversity or richness might be delivered by a completely different set of organisms. We appreciate that metabarcoding isn’t fully quantitative, but the results are often put into semi-quantitative analysis. We are happy with this approach, since the same approach is likely to be employed in interpreting the metabarcoding data from the EES. |
| 19 | What types of pesticides we would be looking at? | Looking at the most recent published pesticide use survey we have identified the following long-list of commonly applied pesticides, and would suggest selecting the test pesticides from this list:   * Fungicides: Tebuconazole; Folpet; Prothioconazole * Herbicides: Glyphosate; diflufenican/flufenacet; fluroxypyr * Insecticides: Lambda-cyhalothrin; Esfenvalerate; Tau-fluvalinate; Pirimicarb * Growth regulator: Chlormequat |